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Regional Variation in the Expression of Bullous Pemphigoid Antigen and Location of Lesions in Bullous Pemphigoid

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The expression of bullous pemphigoid (BP) antigen in different locations on the body was estimated from the highest dilution of BP sera giving a positive reaction at a site by indirect immunofluorescence. Three sera with high titers (640–1280) of BP antibodies against monkey and guinea pig esophagus were reacted with 46 specimens of normal human skin. There was marked variation in the expression of BP antigen in skin obtained from different sites. The greatest expression of BP antigen was in skin obtained from the flexor surfaces of the arm, leg, and thigh. All specimens from these sites reacted with high dilutions (1:640–1:2560) of BP antibodies. BP antigen was expressed least in skin specimens from scalp, face, and extensor surface of the arm. No BP antigen could be demonstrated in 17% of specimens from these locations and the remainder reacted only with low dilutions (1:40–1:160) of BP antibodies. Skin from trunk, knee, and extensor thigh had intermediate amounts of BP antigen. Similar results were seen with all 3 BP sera. Similar reactions were observed in multiple skin specimens obtained from different sites in the same individual. Of note is that the greatest expression of BP antigen was in areas commonly involved in this disease (flexural surfaces), whereas the least was seen in areas (scalp, face, extensor arm) which are rarely involved. The apparent correlation between the expression of BP antigen and the location of lesions in bullous pemphigoid, suggests that BP antigen expression may play a role in the distribution of skin lesions in this disease.

Bullous pemphigoid is a vesiculobullous disease in which lesion production appears to involve autoantibodies to the bullous pemphigoid (BP) antigen [1–5], an antigen present within the lamina lucida in the basement membrane zone of skin [6,7]. Autoantibodies to BP antigen are present in the sera of approximately 70–80% of patients with bullous pemphigoid [8–10]. Although clearly a disease expressing its lesions predominantly in flexural regions of the body [8], no explanation for this geographic preference is known.

This study was undertaken to ascertain whether regional variation in the expression of BP antigen plays a role in the location of clinical lesions in bullous pemphigoid.

MATERIALS AND METHODS

Skin

Studies were perfomed on two sets of normal human skin. Thirty-four specimens were obtained from the edges of surgically excised, benign skin lesions. Another 12 specimens were collected from multiple 4-mm punch biopsies of normal skin in two cadavers taken within 48 h of death. No skin was taken from patients with blistering or autoimmune diseases. All specimens were immediately frozen in liquid nitrogen, cut in 4 μn sections on a cryostat, and tested within 48 h.

Sera

Sera from 3 patients with typical bullous pemphigoid by clinical, histologic, and immunofluorescence criteria were used. The titers of BP antibodies were 640–1280 when tested against monkey and guinea pig esophagus.

Antisera-Conjugate

Fluorescein-labeled goat antihuman IgG conjugate was used (Cappel Labs, Cochranville, Pennsylvania). The concentration of antibody protein was 10 mg/ml, total protein 17.1 mg/ml, and F/P ratio 2.98 mg/g. For use, the conjugate was diluted 1:20 in phosphate-buffered saline (PBS) with 4% bovine salt albumin.

Immunofluorescence Studies

Immunofluorescence studies were performed by standard techniques as previously described [11]. Skin sections were incubated at room temperature with serial serum dilutions. After 30 min, the slides were washed for 10 min in PBS and reincubated for 30 min with fluorescein-labeled conjugate. Specimens were then washed for an additional 30 min in PBS and covered with 0.2 cc glycerin–PBS and examined with a Zeiss binocular microscope equipped with a mercury lamp, fluorescein isothiocyanate exciter and #50 barrier filters.

The expression of BP antigen was estimated from the highest dilution of BP antibody that reacted positively with the specimen (i.e., the end point titer).

All 46 skin specimens were tested with all 3 sera.

RESULTS

There was marked variation in the maximum dilution of bullous pemphigoid sera giving positive reactions when tested against normal skin obtained from different sites in different individuals. The end point titer ranged from 0–2560 when the same BP serum was tested against 46 different specimens of normal human skin (Fig 1).

To examine whether location on the epidermis influenced the expression of BP antigen, the results were reexamined after skin specimens had been grouped according to their location on the body. The results obtained with a single serum are presented in Fig 2 and illustrated in Fig 3*a–h*. The most important observations were that the expression of BP antigen in skin obtained from the same anatomic location in different individuals was similar, whereas it varied markedly in skin obtained from different sites.

The greatest expression of BP antigen, as estimated from the end point titer of BP antibody with skin obtained from that location, was in skin obtained from flexural surfaces (ie., flexor arm, flexor thigh, and popliteal fossa). Thirteen specimens of skin were tested from these sites. All were positive at high dilutions (640–2560) of BP sera. In contrast, the least expression of BP antigen was on the scalp, face, and extensor arm. Eighteen specimens were tested from these sites. No BP antigen could be demonstrated in 3 (17%) specimens. In the remainder, low dilutions (40–160) of BP sera were required to demonstrate BP antigen. Skin from trunk, knee, and extensor thigh had intermediate amounts of BP antigen.

The results obtained with all 3 sera are summarized in Fig 4.

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Abbreviations:

BP: bullous pemphigoid

PBS: phosphate-buffered saline

Although there were small differences in mean titers obtained with the 3 different sera, the general patterns were similar as summarized in Fig 4.

To exclude the possibility that variations in the expression of BP antigen resulted from BP antigen heterogeneity [11] or from variation between individuals rather than between different sites on the body, we analyzed separately the expression of BP antigen in 12 of the skin specimens obtained from different locations in the same individual. Six specimens were obtained from different sites in each of 2 cadavers. The results (Fig 5) confirmed the findings seen in random samples of human skin. The greatest expression of BP antigen was again in flexural surfaces (i.e., popliteal fossa and flexor thigh) and the least on the scalp. The trunk and extensor surface of the extremities showed intermediate expression of antigens. Similar results were obtained with both individuals.

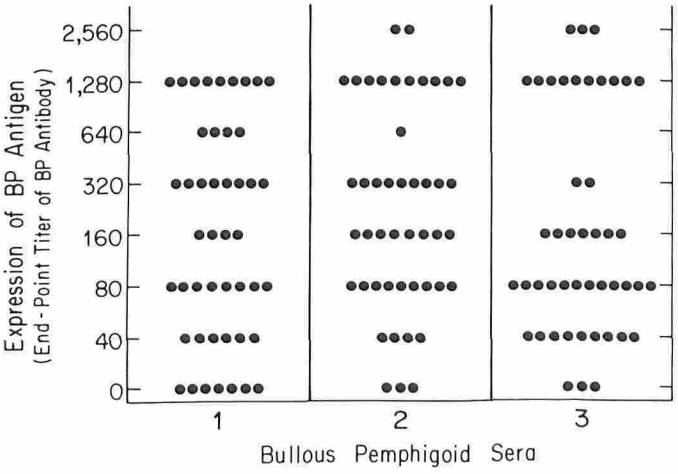

FIG 1. Expression of pemphigoid antigen in different specimens of human skin. Expression of BP antigen was measured from end-point titer of serum containing antibodies to BP antigen. Results obtained with 3 different BP sera are shown.

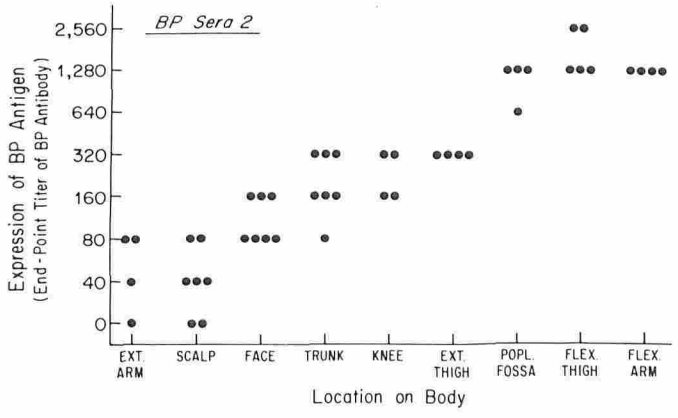

FIG 2. Expression of BP antigen in skin obtained from different sites in different individuals. All specimens tested with same serum (serum 2).

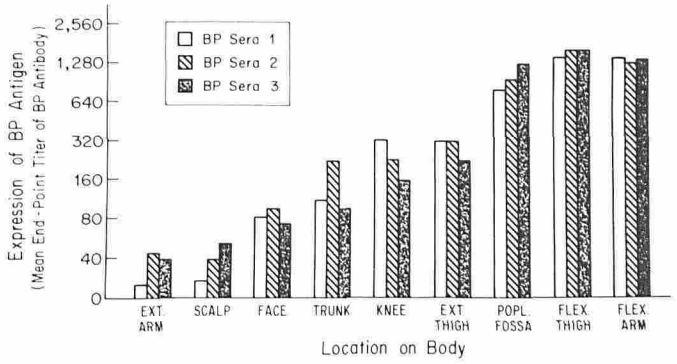

FIG 4. Mean expression of pemphigoid antigen in human skin as a function of location on the body. Each *bar* represents the average BP antigen expression in all skin specimens tested from the indicated area on the body with the same BP serum.

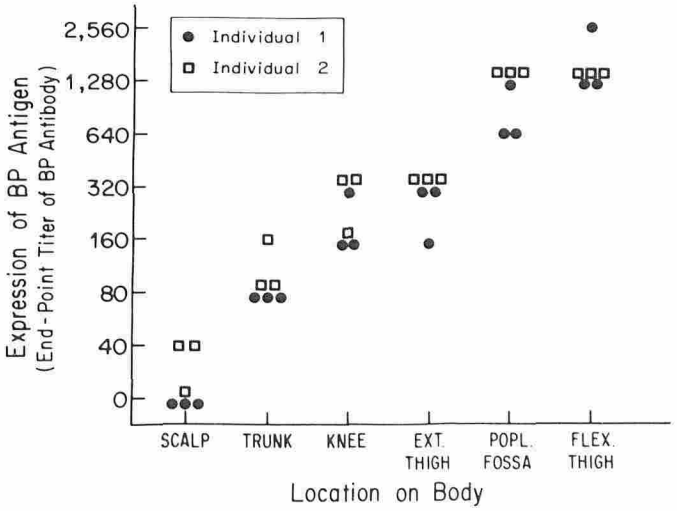

FIG 5. Expression of pemphigoid antigen in same individual. Expression of BP antigen in skin from 6 different sites in the same individual. Results obtained with 2 different individuals each tested with 3 different BP sera.

DISCUSSION

The most important findings of this study are that there is regional variation in the expression of BP antigen in normal human skin and an apparent correlation between the sites of greatest expression of this antigen and the location of lesions in bullous pemphigoid.

Although bullous pemphigoid is commonly accepted to be a

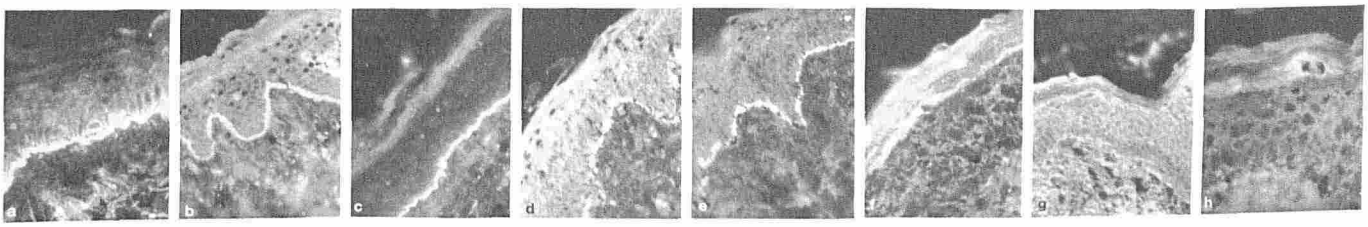

FIG 3. Indirect immunofluorescence photomicrographs of BP antigen expression. Specimens of skin obtained from different sites on the epiderm were reacted with the same BP serum. Photographs all taken at a serum dilution of 1:160. *a*, Popliteal fossa; *b*, flexural thigh; *c*, flexural arm; *d*, knee; *e*, extensor thigh; *f*, face; *g*, scalp; and *h*, extensor arm. There is strong basement membrane zone (BMZ) staining in *a–c*; irregular BMZ staining pattern in *d* and *e*; and absent BMZ staining in *f–h*. (Original magnification × 312.)

disease of autoimmune nature, no reason for the flexural predominance of lesions in this disease has been advanced. In general, very little is known about the factors that influence the distribution of skin lesions in different immune diseases other than allergic contact dermatitis. However, we have previously shown that alterations in the expression of pemphigus antigens in different levels within the epidermis may play a role in bullae location in superficial and deep pemphigus [12,13].

The results of this study show that there is marked regional variation in expression of BP antigen at different sites in human skin. Greatest expression of BP antigen was found in skin from flexural thigh, and flexural and popliteal fossa, sites commonly involved with lesions of bullous pemphigoid. Lesser expression of BP antigens was present on trunk, knees, and extensor thigh, areas of the body less commonly involved in the disease. BP antigen expression was minimal or absent on scalp, face, and extensor arm, rarely involved regions in bullous pemphigoid. These variations were not due to differences in antigen expression among individuals or resulting from BP antigen heterogenicity [11], since specimens obtained from the same location in different individuals all had approximately similar expression of BP antigens. Furthermore, multiple skin specimens from the same individual showed similar changes in BP antigen expression in different regions on the body.

The correlation between expression of BP antigen and location of lesions in this disease strongly suggests that the distribution of lesions in bullous pemphigoid is influenced by regional variations in BP antigen expression.

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